STUDIES ON THE "PRECIPITIN REACTION" IN PLANTS
V. APPLICATION TO PLANT RELATIONSHIPS

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IN AN EARLIER PAPER of this series (2) the senior writer applied the "normal precipitin reaction," better designated by Foster and Avery (5) as the "precipitation reaction," to representatives of a number of families of plants for the purpose of determining whether or not the method is applicable in the study of plant relationships. In most of the material tested at that time there was an abundance of positive reactions, and these tended in general to harmonize with the findings of empirical taxonomy, although a uniformity of negative reactions in some groups limited the procedure to certain families in which well marked positive reactions were obtained. In 1933 Foster and Avery (5) applied a similar technique to the genus *Iris* with results which were satisfying and entirely consistent with those reported in the present series of studies. The purposes of the present paper are to provide additional data on the occurrence of the precipitation reaction to those already recorded, and then from a consideration of all data thus far obtained to analyze the significance of the precipitation reaction in a study of plant relationships with particular reference to the limitations and essential meaning of the reaction. The new groups tested in the present study are the "Amentiferae" and the Guttiferae. The following scheme gives the results of all precipitation tests thus far obtained including the results of the present study.

Family:	*Genera*:	*Tests of genera inter se*:	*Some positive tests obtained with*:	*Entirely negative tests with*:	*Reference*:
Iridaceae	Iris 30[1]	Numerous positive reactions correlated with systematic position.	Solanaceae[2] (CaC_2O_4) Oleaceae Caprifoliaceae Rosaceae "Amentiferae" Guttiferae	Saxifragaceae	Foster & Avery (5); Table IV.[3]

[1]The number refers to the number of species employed in the tests.

[2]Signifies that the only reactions observed were determined as due to calcium oxalate.

[3]Refers to tables in the present paper.

Family:	*Genera*:	*Tests of genera inter se*:	*Some positive tests obtained with*:	*Entirely negative tests with*:	*Reference*:
"Amentiferae"		Uniformly	Solanaceae	Rosaceae	Tables I,
Salicaceae:	Salix 1	negative	Oleaceae	Saxifragaceae	III, IV
Myricaceae:	Myrica 1		Iridaceae	Guttiferae	
Leitneriaceae:	Leitneria 1		Caprifoliaceae		
Juglandaceae:	Carya 1		(CaC_2O_4)		
Betulacaeae:	Alnus 15				
	Betula 16				
	Carpinus 6				
	Corylus 8				
	Ostryopsis 1				
	Ostrya 2				
Fagaceae:	Quercus 1				
Saxifragaceae:	Philadelphus 1	As in	Solanaceae	Iridaceae	Chester (2);
	Fendlera 1	Iridaceae	Rosaceae	"Amentiferae"	table IV
	Schizophragma 1		Platanaceae	Guttiferae	
	Hydrangea 1		Leguminosae		
	Jamesia 1		Oleaceae (CaC_2O_4)		
	Deutzia 1		Caprifoliaceae		
	Itea 1		(CaC_2O_4)		
	Ribes 1				
Platanaceae:	Platanus 1		Solanaceae	Leguminosae	Chester (2)
			Rosaceae		
			Saxifragaceae		
			Oleaceae (CaC_2O_4)		
			Caprifoliaceae (CaC_2O_4)		
Rosaceae:					
Spiroideae:	Spiraea 1		Rosoideae	Pomoideae	Chester (2)
			Prunoideae	Platanaceae	
				Leguminosae	
				Saxifragaceae	
Pomoideae:	Cotoneaster 1	Generally	Iridaceae	Platanaceae	Chester (2);
	Stranvaesia 1	negative	Solanaceae	Leguminosae	table IV
	Chaenomeles 1		Rosoideae	Caprifoliaceae	
	Amelanchier 1		Prunoideae	"Amentiferae"	
	Pyracantha 1		Saxifragaceae	Guttiferae	
	Mespilus 1		Oleaceae		
	Crataegus 1		(CaC_2O_4)		

Family:	*Genera:*	*Tests of genera inter se:*	*Some positive tests obtained with:*	*Entirely negative tests with:*	*Reference:*
Pomoideae: (*continued*)	Sorbus 1 Aronia 1 Photinia 1 Malus 3 Pyrus 1				
Prunoideae:	Prunus 15 Osmaronia 1 Prinsepia 1 Maddenia 1	As in Iridaceae	Saxifragaceae Leguminosae Pomoideae Platanaceae Solanaceae Oleaceae (CaC_2O_4) Caprifoliaceae (CaC_2O_4)	Iridaceae "Amentiferae" Guttiferae	Chester (2); table IV
Leguminosae:	Robinia 1		Solanaceae Rosaceae Saxifragaceae Oleaceae (CaC_2O_4) Caprifoliaceae (CaC_2O_4)	Platanaceae	Chester (2)
Guttiferae:	Hypericum 10	Uniformly negative	Saxifragaceae Solanaceae Oleaceae Iridaceae Caprifoliaceae (CaC_2O_4) Rosaceae	"Amentiferae"	Tables II, III, IV
Oleaceae:	Syringa 2 Ligustrum 8 Fraxinus 1 Chionanthus 1 Forsythia 1	Uniformly negative except for calcium oxalate reaction	Solanaceae Iridaceae "Amentiferae" Guttiferae Rosaceae (CaC_2O_4) Saxifragaceae (CaC_2O_4) Caprifoliaceae (CaC_2O_4) Platanaceae (CaC_2O_4) Leguminosae (CaC_2O_4)		Chester (1); table IV

Family:	*Genera*:	*Tests of genera inter se*:	*Some positive tests obtained with*:	*Entirely negative tests with*:	*Reference*:
Solanaceae:	Lycopersicum 1 Salpiglossis 1 Cyphomandra 1 Browallia 1 Nicotiana 19 Capsicum 2 Solanum 5 Physalis 1 Petunia 1 Lycium 1 Datura 4 Atropa 1	As in Iridaceae	Oleaceae Iridaceae (CaC_2O_4) Rosaceae Saxifragaceae Caprifoliaceae Platanaceae Leguminosae "Amentiferae" Guttiferae		Kostoff (6); Chester & Whitaker (3); table IV
Caprifoliaceae:	Kolkwitzia 1 Symphoricarpus 1 Diervilla 1 Viburnum 1 Linnaea 1 Sambucus 1 Dipelta 1 Abelia 1 Lonicera 2	Uniformly negative	Solanaceae Iridaceae Rosaceae Guttiferae Oleaceae (CaC_2O_4) Saxifragaceae (CaC_2O_4) Platanaceae (CaC_2O_4) Leguminosae (CaC_2O_4) "Amentiferae" (CaC_2O_4)		Chester (2); table IV

Thus up to the present fifteen families and approximately two hundred species of plants have been tested more or less extensively with regard to the precipitation reaction. Of these fifteen families, four (Solanaceae, Iridaceae, Saxifragaceae and Rosaceae-Prunoideae) have yielded among themselves significant positive results from the taxonomic standpoint. In five of the other eleven groups (Oleaceae, Rosaceae-Pomoideae, Caprifoliaceae, Guttiferae, "Amentiferae") fairly extensive tests within the groups have yielded wholly negative results, while in the remaining seven families the results thus far obtained are inadequate for sound conclusions because the number of species tested is too limited.

The present paper reports the results obtained in tests of the precipitation reactions of forty-five species of Betulaceae, one species each of five other families of "Amentiferae," ten species of *Hypericum* (Guttiferae), and two species each of the Iridaceae, Solanaceae, Oleaceae, Rosaceae, Caprifoliaceae, and Saxifragaceae. The technique employed

TABLE I. PRECIPITATION REACTIONS IN THE BETULACEAE

"t"...trace; "1"...weak reaction; "2"...moderate reaction; "3"... strong reaction; "O"... no reaction. Experiments performed with dried herbarium material.

	Alnus crispa #1.	A.firma	Betula nigra	B.pumila	Carpinus Japonica	C.Turczaninowii	C.cordata	C.orientalis	C.betulus	Betula grossa	B.Schmidtii	B.pendula	B.coerulea-grandis	B.Maximowicziana	B.populifolia	Corylus heterophylla	C.avellana	C.Sieboldiana	C.maxima	Alnus Spaethii	A.maritima	A.tenuifolia	Corylus americana	Betula papyrifera	Alnus hirsuta	A.incana	A.japonica	A.rugosa	Betula Jackii	B.japonica	B.medwediewii	B.papyrifera occident.	B.globispica	Carpinus caroliniana	Corylus cornuta	C.spinescens	Ostryopsis Davidiana	O.japonica	O.virginiana	Alnus rubra	Betula nigra	Alnus rhombifolia	A.orientalis	A.subcordata	A.tenuifolia	Betula glandulosa	B.neoalaskana	Alnus crispa #2.
Alnus crispa #1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
A.firma	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Betula nigra	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
B.pumila	0	0	0	0		0	0	0	0				0			0	0	0		0	0	0																										
Carpin.japon.	0	0	0		0	0	0	0	0				0			0	0	0	0		0																											
C.Turczaninowii	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
C.cordata	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
C.orientalis	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
C.betulus	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Betula grossa	0	0	0			0	0	0	0	0			0																																			
B.Schmidtii	0	0	0			0	0	0	0	0			0			0	0	0		0	0	0	0		0	0	0			0	0	0		0	0	0	0											
B.pendula	0	0	0			0	0	0	0			0	0			0	0	0		0	0																											
B.Coer.-grandis	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
B.Maximowicziana	0	0	0			0	0	0	0				0	0		0	0	0		0	0	0	0		0	0	0			0	0	0		0	0	0	0	0	0	0	0	0	0	0	0		0	
B.populifolia	0	0	0			0	0	0	0				0		0	0																																
Corylus heteroph.	0	0	0	0	0	0	0	0	0		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
C.avellana	0	0	0	0	0	0	0	0	0		0	0	0	0		0	0	0	0	0	0	0	0	0	0	0	0	0		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
C.Sieboldiana	0	0	0	0	0	0	0	0	0		0	0	0	0		0	0	0	0	0	0	0	0	0	0	0	0	0		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
C.maxima	0	0	0		0	0	0	0	0				0			0	0	0	0	0	0	0	0	0	0	0	0			0	0	0		0	0	0	0											
Alnus Spaethii	0	0	0	0	0	0	0	0	0		0	0	0	0		0	0	0	0	0	0	0	0	0	0	0	0	0		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
A.maritima	0	0	0	0	0	0	0	0	0		0	0	0	0		0	0	0	0	0	0	0	0	0	0	0	0	0		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
A.tenuifolia	0	0	0	0		0	0	0	0		0		0	0		0	0	0	0	0	0	0	0	0	0	0	0	0		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		0	0
C.americana	0	0	0			0	0	0	0		0		0	0		0	0	0	0	0	0	0	0	0	0	0	0	0		0	0	0		0	0	0	0	0	0	0	0	0	0	0	0		0	0
Bet.papyrifera	0	0	0			0	0	0	0				0			0	0	0		0	0	0	0	0	0	0	0	0		0	0	0		0	0	0	0											
Alnus hirsuta	0	0	0			0	0	0	0		0	0	0	0		0	0	0	0	0	0	0	0	0	0	0	0	0		0	0	0		0	0	0	0	0	0	0	0	0	0	0	0		0	0
A.incana	0	0	0			0	0	0	0		0		0	0		0	0	0	0	0	0	0	0	0	0	0	0	0		0	0	0		0	0	0	0	0	0	0	0	0	0	0	0		0	0
A.japonica	0	0	0			0	0	0	0		0		0	0		0	0	0	0	0	0	0	0	0	0	0	0	0		0	0	0		0	0	0	0	0	0	0	0	0	0	0	0		0	0
A.rugosa	0	0	0			0	0	0	0				0			0	0	0		0	0	0	0	0	0		0	0		0	0	0		0	0	0	0	0	0	0	0							
Betula Jackii																																																
B.japonica	0	0	0			0	0	0	0		0		0	0		0	0	0	0	0	0	0	0	0	0	0	0	0		0	0	0		0	0	0	0	0	0	0	0	0	0	0	0		0	0
B.Medwediewii	0	0	0			0	0	0	0		0		0	0		0	0	0	0	0	0	0	0	0	0	0	0	0		0	0	0		0	0	0	0	0	0	0	0	0	0	0	0		0	0
B.pap.occident.	0	0	0			0	0	0	0		0		0	0		0	0	0	0	0	0	0	0	0	0	0	0	0		0	0	0		0	0	0	0	0	0	0	0	0	0	0	0		0	0
B.globispica	0	0	0			0	0	0	0				0			0	0	0		0	0	0																										
Carp.caroliniana	0	0	0			0	0	0	0		0		0	0		0	0	0	0	0	0	0	0	0	0	0	0	0		0	0	0		0	0	0	0	0	0	0	0	0	0	0	0		0	0
Cory.cornuta	0	0	0			0	0	0	0		0		0	0		0	0	0	0	0	0	0	0	0	0	0	0	0		0	0	0		0	0	0	0	0	0	0	0	0	0	0	0		0	0
C.spinescens	0	0	0			0	0	0	0		0		0	0		0	0	0	0	0	0	0	0	0	0	0	0	0		0	0	0		0	0	0	0	0	0	0	0	0	0	0	0		0	0
Ostryop.David.	0	0	0			0	0	0	0		0		0	0		0	0	0	0	0	0	0	0	0	0	0	0	0		0	0	0		0	0	0	0	0	0	0	0	0	0	0	0		0	0
O.japonica	0	0	0			0	0	0	0				0	0		0	0	0		0	0	0	0		0	0	0	0		0	0	0		0	0	0	0	0	0	0	0	0	0	0	0		0	0
O.virginiana	0	0	0			0	0	0	0				0	0		0	0	0		0	0	0	0		0	0	0	0		0	0	0		0	0	0	0	0	0	0	0	0	0	0	0		0	0
Alnus rubra	0	0	0			0	0	0	0				0	0		0	0	0		0	0	0	0		0	0	0	0		0	0	0		0	0	0	0	0	0	0	0	0	0	0	0		0	0
Betula nigra	0	0	0			0	0	0	0				0	0		0	0	0		0	0	0	0		0	0	0	0		0	0	0		0	0	0	0	0	0	0	0	0	0	0	0		0	0
Al.rhombifol.	0	0	0			0	0	0	0				0	0		0	0	0		0	0	0	0		0	0	0			0	0	0		0	0	0	0	0	0	0	0	0	0	0	0		0	0
A.orientalis	0	0	0			0	0	0	0				0	0		0	0	0		0	0	0	0		0	0	0			0	0	0		0	0	0	0	0	0	0	0	0	0	0	0		0	0
A.subcordata	0	0	0			0	0	0	0				0	0		0	0	0		0	0	0	0		0	0	0			0	0	0		0	0	0	0	0	0	0	0	0	0	0	0		0	0
A.tenuifolia	0	0	0			0	0	0	0				0	0		0	0	0		0	0	0	0		0	0	0			0	0	0		0	0	0	0	0	0	0	0	0	0	0	0		0	0
B.glandulosa	0	0	0			0	0	0	0				0			0	0	0		0	0																											
B.neoalaskana	0	0	0			0	0	0	0				0	0		0	0	0		0	0	0	0		0	0	0			0	0	0		0	0	0	0	0	0	0	0	0	0	0	0		0	0
Alnus crispa #2.	0	0	0			0	0	0	0				0			0	0	0		0	0	0	0		0	0	0			0	0	0		0	0	0	0	0	0	0	0	0	0	0	0		0	0
Calcium chloride	3	t	1	t	1	2	1	3	1	1	1	1	1	1	2	2	1	2	1	t	1	2	1	t	1	3	2	2	2	1	t	1	1	2	1	2	1	1	3	2	1	2	1	2	2	1	2	t
Potass.oxalate	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

was essentially as described in the earlier papers of this series (1, 2). In testing the majority of the Betulaceae dried herbarium specimens were used for material, and these tests were later confirmed by repetition of many of them using fresh leaves of the same plants. The tests of the other "Amentiferae" and other families mentioned above were performed with freshly collected leaves. The dried leaves were extracted for a few hours in ten times their weight of distilled water, and

TABLE II. PRECIPITATION REACTIONS IN HYPERICUM (GUTTIFERAE)

"t"...trace; "+"...weak reaction; "++"... moderate reaction; "+++"...strong reaction; "O"...no reaction. Experiments performed with fresh material.

	Hypericum Ascyron #1.	H.galioides	H.aureum	H.arnoldanum	H.Kalmianum	H.nudiflorum	H.lobocarpum	H.Buckleyi	H.boreale	H.perforatum	H.Ascyron #2.	Potass.oxalate	Calcium chloride
Hypericum Ascyron #1.	O	O	O	O	O	O	O	O	O	O	O	+	O
H.galioides	O	O	O	O	O	O	O	O	O	O	O	t	O
H.aureum	O	O	O	O	O	O	O	O	O	O	O	+	O
H.arnoldanum	O	O	O	O	O	O	O	O	O	O	O	t	O
H.Kalmianum	O	O	O	O	O	O	O	O	O	O	O	+	O
H.nudiflorum	O	O	O	O	O	O	O	O	O	O	O	t	O
H.lobocarpum	O	O	O	O	O	O	O	O	O	O	O	++	O
H.Buckleyi	O	O	O	O	O	O	O	O	O	O	O	O	O
H.boreale	O	O	O	O	O	O	O	O	O	O	O	t	O
H.perforatum	O	O	O	O	O	O	O	O	O	O	O	+	O
H.Ascyron #2.	O	O	O	O	O	O	O	O	O	O	O	+	O

the fresh leaves in three to four times their weight of distilled water. The results of these tests are given in Tables I, II, III, and IV below.

As is indicated in Table I an experiment involving forty-five species of the Betulaceae showed no positive results. These species represent every section of every genus of the family as recognized by Winkler (8) with the exception of the section Cremastogyne in *Alnus*. In every case the material used was authenticated by herbarium specimens from which dried leaves were obtained for the tests. Through the kindness of Mr. Rehder of the Arnold Arboretum the determinations of these

TABLE III. PRECIPITATION REACTIONS IN THE "AMENTIFERAE"

Notations as in Table II. Experiments performed with fresh material.

	Ostryopsis	Ostrya virginiana	Carpinus cordata	Carpinus caroliniana	Corylus cornuta	Betula nigra	B.pumila	B.occidentalis	B.Maximowicziana	Alnus crispa	A.incana	Quercus alba	Leitneria floridana	Salix Matsudana	Carya alba	Myrica	Hypericum nudiflorum	Potass.oxalate.	Calcium chloride
Ostryopsis	0	0	0	0	0	0	0	0	0	0	0	0	0		0	0	0	++	0
Ostrya virginiana	0	0	0	0	0	0	0	0	0	0	0	0	0		0	0	0	+	0
Carpinus cordata	0	0	0	0	0	0	0	0	0	0	0	0	0		0	0	0	+	0
C.caroliniana	0	0	0	0	0	0	0	0	0	0	0	0	0		0	0	0	+	0
Corylus cornuta	0	0	0	0	0	0	0	0	0	0	0	0	0		0	0	0	+	0
Betula nigra	0	0	0	0	0	0	0	0	0	0	0	0	0		0	0	0	+	0
B.pumila	0	0	0	0	0	0	0	0	0	0	0	0	0		0	0	0	+	0
B.occidentalis	0	0	0	0	0	0	0	0	0	0	0	0	0		0	0	0	+	0
B.Maximowicziana	0	0	0	0	0	0	0	0	0	0	0	0	0		0	0		+	0
Alnus crispa	0	0	0	0	0	0	0	0	0	0	0	0	0		0	0	0	++	0
A.incana	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	+	0
Quercus alba	0	0	0	0	0	0	0	0	0	0	0	0	0		0	0	0	++	0
Leitneria florid.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	++	0
Salix Matsudana											0		0			0	0	++	0
Carya alba	0	0	0	0	0	0	0	0	0	0	0	0	0		0	0	0	+	0
Myrica	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	+	0
Hypericum nudifl.	0	0	0	0	0	0	0	0		0	0	0	0	0	0	0	0	t	0

specimens were checked and the specific names are cited in accordance with his treatment of the family in his "Manual of Cultivated Trees and Shrubs" (7).

In view of the discrepancy observed in *Iris* by Foster and Avery (5) between the results obtained with fresh as contrasted with dried leaves, it was thought advisable to check the observations presented in Table I using fresh leaves from certain of the same plants involved in the tests previously made. Opportunity was also taken to introduce one species each of the following families: Fagaceae, Leitneriaceae, Salicaceae, Juglandaceae, and Myricaceae. These other families of the "Amentiferae" were introduced in the hope that, should differences of reactivity within the group occur, some light might be shed on the vexing question of relationships between these families. A member of the Guttiferae was employed as a check on possible wider relationships. Again the observations (Table III) show absence of reactivity between the leaf tissue extracts.

To determine what reactive substances occur in the Betulaceae, in the Leitneriaceae, and in *Hypericum* of the Guttiferae, the concept of "test plant" of a "known" constitution was introduced. Fresh material of the species with the label-names indicated in Table IV was collected in the Arnold Arboretum and these were tested against each other. The results indicate a similar reactivity in *Betula populifolia, Diervilla florida,* and *Malus Arnoldiana,* since these show positive reactions only with *Iris chrysophoenicia, Nicotiana alata,* and *Lycopersicum esculentum,* and all contain an excess of calcium ion. This suggests the presence of but one principle of a reactive pair, probably "A" (3), since the *Nicotiana alata* and *Lycopersicum esculentum* have been shown in an earlier paper (3, p. 186, fig. IV) to contain an excess of the complement "B." The possibility of this being the "MN" reaction (l. c.) is eliminated because of the negative reactions with *Ligustrum obtusifolium* which is known from the earlier work to be "N+."

An interesting situation exists in the case of *Leitneria* where but one odd reaction occurred, namely with *Syringa velutina,* suggestive of a fifth reactive pair. Further investigation is needed here.

An analysis of the results presented in Tables I and III yields little of value in the light of these conclusions, beyond the fact that opposing members of reactive pairs are absent. One of these is probably "B" and another the counterpart of the unknown existing in *Leitneria.* This striking absence of reactivity within the Betulaceae might well be construed in confirmation of preexisting morphological evidence indicating close relationship between the members of the family. But in considering the "Amentiferae" with their similar negative reactions, it should be kept in mind that widely varying treatments of the relationships between the families composing this group exist. Our evidence, in the light of the hypothesis expressed in an earlier paper (2) and later accepted by Foster and Avery (5), i. e. that negative results indicate very close or very distant relationship, helps not at all in clarifying the inter-relationships of the "Amentiferae."

The genus *Hypericum* as represented here includes ten species of five different sections as recognized by R. Keller in Engler and Prantl (4). The species vary from the small herbaceous *H. boreale* (Britton) Bicknell, the larger herbaceous *H. perforatum* L. and *H. Ascyron* L., through the shrubby forms to the dwarf mountain shrub *H. Buckleyi* M. A. Curtis. The list includes one known hybrid *H. Arnoldianum* Rehder with both parents (*H. galioides* and *H. lobocarpum*) (7). All the species used were collected in the Arnold Arboretum and authenticated by the junior author.

The precipitation reactions in *Hypericum* show consistent negative results as may be seen in Table II. From this one may conclude that the genus is homogeneous regarding reactive substances or that they are absent. In Table III one species of *Hypericum* tested against the "Amentiferae" shows no positive reactions. This affords no clue to the situation. However, when tested against other representative families (Table IV) it will be seen that the species is highly reactive. Unfortunately a wide enough variety of "known" plants was not used in the present study to make a complete analysis possible. It will be seen (Table IV) that good positive reactions were obtained with *Iris chrysophoenicia* and with the solanaceous representatives, *Nicotiana alata* and *Lycopersicum esculentum*. Since these species did not react against each other but reacted consistently against other forms, one may conclude the reaction to be of similar quality and doubtless due to the presence of the same reactive substances. The reactions with the oleaceous members, *Syringa velutina* and *Ligustrum obtusifolium*, must be due to a second set of reactive principles, since the Oleaceae also reacted with the Solanaceae. In the same manner the positive reaction with *Lonicera Myrtillus* probably represents a third set of characters. The trace shown with *Philadelphus grandiflorus* is of doubtful character since this species did not react with any other plant, thus giving no indication of the reactive substances involved.

From the evidence presented one is justified in concluding the presence of three reactive principles in *Hypericum;* whether these fall into the categories of the reactive substances designated in an earlier paper (2) as "AB," "MN," and "XY" is not known. Although we cannot be sure that the same principles occur in all species of *Hypericum,* at least the species tested do not show the presence of any of the opposing characters. The known reaction, calcium-oxalate, is of no significance in indicating degree of relationship in this genus as is shown by the harmony of negative results obtained when tested against calcium chloride. Thus it would seem that in the genus *Hypericum* the precipitation technique would be of little value in determining relationships within the group.

TABLE IV. PRECIPITATION REACTIONS IN REPRESENTATIVES OF ALL THE FAMILIES THUS FAR INTENSIVELY STUDIED WITH REGARD TO THE PRECIPITATION REACTION

Notations as in Table II. Experiments performed with fresh material. Extracts containing an excess of oxalate neutralized with calcium chloride and re-filtered before testing.

	Iris pseudacorus	I. chrysophoenicia	Nicotiana alata	Lycopersicum esculenta	Syringa velutina	Ligustrum obtusifolium	Malus Arnoldiana	Prunus serrulata	Philadelphus grandiflorus	Deutzia scabra plena	Diervilla florida	Lonicera myrtillus	Leitneria floridana	Betula populifolia	Hypericum lobocarpum	Calcium chloride .01M	Potass. oxalate .012M
Iris pseudacorus	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	+
I. chrysophoenicia	0	0	0	0	++	+	+	0	0	0	+	++	0	++	+++	0	++
Nicotiana alata	0	0	0	0	+++	+	+	0	0	0	+	++	0	++	+++	0	+++
Lycopersicum es.	0	0	0	0	++	+	+	0	0	0	+	+	0	+	++	+	0 (++)
Syringa velutina	0	++	+++	++	0	0	0	0	0	0	0	0	+	0	++	+++	0 (+++)
Ligustrum obtus.	0	+	+	+	0	0	0	0	0	0	0	0	0	0	++	0	++++
Malus Arnoldiana	0	+	+	+	0	0	0	0	0	0	0	0	0	0	0	0	+++
Prunus serrulata	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	+++
Philadelphus gr.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	t	0	0
Deutzia scabra	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	+++
Diervilla florida	0	+	+	+	0	0	0	0	0	0	0	0	0	0	0	0	+++
Lonicera myrtil.	0	++	++	+	0	0	0	0	0	0	0	0	0	0	+	+++	0 (++)
Leitneria florid.	0	0	0	0	+	0	0	0	0	0	0	0	0	0	0	0	+
Betula populifol.	0	++	++	+	0	0	0	0	0	0	0	0	0	0	0	0	++
Hypericum lobocarp.	0	+++	+++	+++	++	++	0	0	t	0	0	+	0	0	0	0	+++

We are now in a position to resolve more satisfactorily the question of the taxonomic significance of the precipitation reaction in plants. Within certain of the limited plant groups tested (families, subfamilies, and genera) there have been obtained numerous positive reactions.

These positive reactions show a marked correlation with the taxonomic positions of the plants involved (2, 3, 5). Within others of the limited groups tested (Caprifoliaceae, Oleaceae, "Amentiferae," Guttiferae) entirely negative results have been obtained. In at least three of these (Oleaceae, Betulaceae, and Guttiferae) the genera and species tested are usually considered rather closely related to one another and the negative findings may well be of significance indicative of this close relationship. In such groups as the Caprifoliaceae, which while rather heterogeneous still display uniformly negative reactions, one may only conclude that chemical differences demonstrated by the precipitation technique are not necessarily associated with gross morphological differences, since any category of characters used for classification may vary independently of any other, a conclusion consistent with the findings whenever any two techniques (e. g. morphology, cytology, anatomy, genetics, etc.) are used in a taxonomic study of a given group of plants.

As regards systematics on a larger scale, that is, phylogeny of the angiosperms taken as a whole, the precipitation technique is apparently not applicable in a differentiation of widely separated groups. This is evident when one considers Table IV which gives the results of inter-family tests of nine widely separated families of plants. Here no useful correlation of precipitation reaction with systematic position is to be seen. The reason for this is quite apparent when one considers the nature of the precipitation reaction.

The thousands of positive reactions which have been observed in fifteen families of plants do not represent thousands of specific reactions but rather represent a relatively small number of analyzable reactions, one of which has been chemically determined and three of which have to some extent been characterized. Two or three pairs of reactive substances suffice to account for all of the inter-family reactions of Table IV; four pairs of reactive substances accounted for all of the reactions of paper III of this series (Solanaceae et al.). The substances responsible for any given reaction seem to be rather widely distributed throughout the angiosperms. Thus (Table IV) the substance in the Solanaceae which reacts with oleaceous extracts is likewise present in *Iris*, and the oleaceous complement also in *Malus* and the Caprifoliaceae. This fact does not detract from the value of the precipitation reaction when confined to limited groups of plants (families, subfamilies, genera). Within such limited groups it has been found that morphologically similar species tend to carry similar complements of precipitating substances (2, 3, 5).

The value of the technique within families of the higher plants when used to determine similarity or difference as far as the three or more unknown and one known pairs of variables are concerned, is to be evaluated as in the case of any other category of evidence with a similar number of variables. If all four or more pairs of variables are present in a given family the technique is correspondingly significant. If the number of variables is less, the value of the technique is accordingly reduced, until we reach a condition such as exists in the Betulaceae and apparently the "Amentiferae" as a whole where the precipitation technique indicates no difference between the species tested.

It is obvious from an examination of the experimental data thus far obtained that the method in its present form with only a relatively few pairs of variables provides hardly enough combinations of characters to be of significant aid in determining the extent of relationship between widely separated families. Thus we must guard against a possible misinterpretation of the statement made earlier in this series of papers (2) and later confirmed by Foster and Avery (5) that absence of reaction indicates very close or very distant relationship, while a positive reaction indicates an intermediate degree of relationship. Clearly this concept cannot be applied to groups too diverse morphologically—its use must be restricted to plants closely related as determined by other means. Within such groups it should be kept in mind that the applicability of the method is determined by the number of distinct reactive pairs of substances. The greater the number of these the greater the number of categories into which the plants being investigated can be placed.

In pointing out this limitation of the applicability of the precipitation reaction in plant systematics let us compare it with the use of chromosome number in taxonomy. Within limited groups of plants chromosome number may aid in classifying species; the fact that the same chromosome number may be found in very distantly related plant groups does not detract from the use of chromosome number in plant classification. The same is true of the precipitation reaction; its applicability in closely related groups of plants is not to be belittled by the fact that the same reactive substance may occur in very distantly related plant families.

The work done up to the present on the precipitation reaction in plants marks only a groping beginning toward a phase of plant systematics which will doubtless develop more widely in years to come, namely, the use of chemical properties in the study of plant relationships. In testing plants by the precipitation technique one is actually testing related groups of species against arbitrary and fortuitously selected "test extracts." Since there is apparently only a very limited number of types of precipitation reaction present in plants, one would actually gain in time and efficiency if he were to select a few "test species" containing various known reactive complements, and use these as standards against which to test all species of selected limited groups. That this "test species" concept has its limitations is shown by a comparison of the reactivity observed in Table IV with that reported by Foster and Avery and in previous papers of this series. The reaction indicated between *Iris chrysophoenicia* and *I. pseudacorus* is strictly negative according to our results but (+ +) according to Foster and Avery. Similar discrepancies exist in the behavior of *Solanum lycopersicum* and *Nicotiana alata* which in work earlier reported (3) were negative to oleaceous forms but were found (Table IV) in this series of experiments to be positive. It would seem that in order to define the constitution of an unknown plant according to the suggestion made in paper III of his series (p. 185), it is necessary in every new series of experiments to redetermine critically the constitution of "test extracts."

To go one step further, would it not be more satisfactory to submit the few reactions found to detailed biochemical analysis in order eventually to substitute for the "test species" simple chemical solutions of known composition, containing only the active ingredients of the "test extracts"? The senior writer has already done this with respect to one, namely the calcium oxalate reaction. A relatively small number of such solutions would enormously simplify the precipitation technique and would give results far more accurate and explainable than those thus far obtained. The inquiring botanist at this point must turn to the skilled biochemist for aid in resolving this problem. It is essential now that the precise nature of the precipitation reactions in plants be made the subject of investigations by someone adequately trained in analytical organic chemistry; the results of his research could not fail to be of value in advancing our knowledge of this phase of plant systematics.

LITERATURE CITED

1. CHESTER, K. S. (1931). Graft-blight: A disease of Lilac related to the employment of certain understocks in propagation. Jour. Arnold Arb. **12**:79–146.
2. ———— (1932). Studies on the precipitin reaction in plants. I. The specificity of the normal precipitin reaction. Ibid. **13**:52–74.

3. ———— and T. W. Whitaker (1933). Studies on the precipitin reaction in plants. III. A biochemical analysis of the "normal precipitin reaction." Ibid. **14**:118–197.
4. Engler, A. and K. Prantl (1895). Die natürlichen Pflanzenfamilien, usw. III. Teil, Abt. 6 und 6a. Leipsig, W. Engelmann.
5. Foster, R. C. and G. S. Avery (1933). Parallelism of precipitation reactions and breeding results in the genus *Iris*. I. Preliminary study and correlation with other evidence. Bot. Gaz. **94**:714–728.
6. Kostoff, D. (1929). Acquired immunity in plants. Genetics, **14**:37–77.
7. Rehder, A. (1927). Manual of cultivated trees and shrubs. New York, Macmillan.
8. Winkler, H. (1904). Betulaceae in Das Pflanzenreich, IV, 61.

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